Electronic Supplementary Material (ESI) for Lab on a Chip.

Investigating the fluid dynamics of rapid processes within microfluidic devices using bright-field microscopy

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Supplementary Information

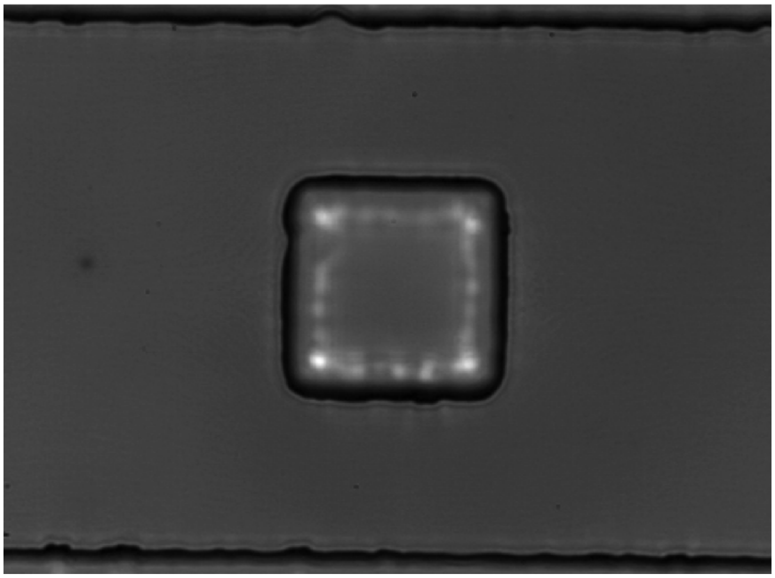

Figure S1: Image obtained by extracting the median of 20 successive images of the flow of DI water seeded with 200 nm polystyrene particles past a square obstacle (80 µm × 80 µm) within a microchannel. The volumetric flow rate was 10 µl/min with images captured at 10, 000 frames per second and a 12 µs exposure time.

Materials and Methods

Microfluidic devices were structured in polydimethylsiloxane (Sylgard 184, Dow Corning) and fabricated using standard soft lithographic methods. Briefly, a thin layer of a photoresist resin, SU-8 (MicroChem, Newton, USA), was spin-coated on a silicon wafer and the final master design obtained by UV exposure through a photomask designed using AutoCAD software (Autodesk Inc., San Rafael, CA). Finally, PDMS was poured on the molds and cured at 70˚C for 2 hours to create a negative replica. The PDMS substrate was then bonded to a planar glass surface by exposing both surfaces to a corona discharge (ETP, Chicago, IL, USA).

For the generation of water-in-oil droplets a fluorinated oil (FC-40, 3M, Sigma, Poole, UK) was used as the continuous phase and deionized water seeded with 200 nm polystyrene particles (Sigma Aldrich, Poole, UK) used as the dispersed phase. 2% Raindance surfactant (RainDance Technologies, MA, USA) was added into the continuous phase to stabilize the droplets after formation. Syringe pumps (Harvard Apparatus Inc., South Natick, USA) were used to control volumetric flow rates of all input streams. Images were analysed using ImageJ software (NIH, Bethesda, USA).

Ghost Particle Velocimetry

A Nikon Ti-E inverted microscope was operated under Koehler illumination conditions and the numerical aperture of the condenser, NA_c, was set to 0.15. Reduction of the numerical aperture of the condenser generates a partially coherent illumination that affects only a finite layer of the sample in the vertical direction. The thickness of the sampled layer, δ_z, depends both on the numerical aperture of the condenser and on the wavelength of the light, λ, as described in Buzzaccaro *et al.*[1], i.e. $\delta_z \sim \lambda/(NA_c)^2$. For our operating conditions, this corresponds to a region of the sample 25 μm thick.

Since the size of a single speckle must be bigger that 2 pixels to be distinguishable from the camera noise, we adopted a higher magnification objective. It was found that for the current experimental conditions a 20X (or higher) objective satisfies this requirement, whilst a 10X (or lower) magnification objective does not. When measuring the velocity fields associated with rapid phenomena, data must be collected at a fast rate and thus the frame rate must be proportional to the flow field. In particular, the MATLAB code used to analyse the images (*PIVlab*) considers regions of interest (ROIs) for the correlation of each frame with the next. When choosing the optimal frame rate it is essential to consider the size of the interrogation area. The latter should be at least twice as big as the largest detectable displacement in a sequence of two consecutive frames to guarantee sufficient spatial resolution to map the velocity field. Indeed, *PIVlab* performs cross correlation between each pair of images by considering a displacement, from frame to frame, equal to 50% of the ROI's size.

To match the velocity field in the droplet formation experiments, where the linear velocities exceed 50 mm/s, movies were recorded at up to 15,000 fps, using an exposure time as low as 10 μs. To increase the resolution of the velocimetry measurements, a three-pass technique was employed (and implemented in *PIVlab* software), where each step employs a ROI smaller than the previous one. The results obtained with each pass are used as first input for the second iteration.

The accuracy of the velocity data presented depends on the region of interest (ROI) over which we mediate our GPV analysis, the objective magnification and the camera pixel size. For example, in both Figures 4 and 5, we used a ROI as small as 18 pixels x 18 pixels, a 60X objective and a camera pixel size of 20 μm. Thus converted to real space units the ROI size corresponded to approximately 7 x 7 μm^2. Each velocity value was hence obtained from the average of the velocity field over each ROI and then plotted, as a color-coded map, to generate the data presented in Figures 4 and 5.

Averaging techniques for the velocimetry of droplet formation using *PIVlab* software

When studying the velocity field inside the dispersed phase during droplet formation we considered each droplet to be identical. This assumption allows consideration of a sequence of two frames depicting the same event for several droplets; the velocity fields obtained from each pair of images were then averaged together to obtain a better signal-to-noise ratio. This technique was used to generate Figure 4 by considering 10 subsequent droplets. The sequence of the two images for each of the 10 droplets (a total of 20 frames) is shown in the Movie S4.

After the neck breaks and the water droplet is released into the oil phase the dynamics of mixing inside the droplet were probed. We recorded images at 15,000 fps and analysed the flow dynamics inside the droplet by changing the frame of reference. The latter was obtained by following a single droplet in the microchannel and by image manipulation via a custom Matlab routine. We used this technique to obtain Figure 5 in which we consider 20 consecutive frames and average the velocity field obtained from each pair of images over the whole dataset to minimize noise due to spurious effects. We assumed a constant hydrodynamic behaviour inside the droplet over the 20 frames (equivalent to 1.33 ms). The complete sequence of images is included as Supplementary Movie 5.

Movie captions

Movie S1

200 nm polystyrene particles dispersed in DI water flowing in a microchannel (200 μm wide and 50 μm high) with a square pillar (80 μm × 80 μm) in the middle. The flow rate is 10 μl/min and the flow direction is from left to right. The movie was captured at 10,000 frames per second with a 12 μs exposure time.

Movie S2

Speckle patterns obtained by subtraction of the median image (see Figure S1) from each frame of Movie S1.

Movie S3

Water-in-oil droplet generation; the flow rates are 10 μl/min for the oil and 2 μl/min for the aqueous phase. The movie was captured at 15,000 frames per second with a 12 μs exposure time.

Movie S4

Speckle patterns used to generate Figure 4 (c) with *PIVlab* software. The speckle pattern of two consecutive frames from 10 successive droplet generation events is used.

Movie S5

Relative movement of the speckle pattern inside a droplet moving from right to left. Figure 5 was realized by averaging the velocity field obtained by analyzing 2 by 2 the frames of this movie with *PIVlab* software. The movie was recorded at 15,000 fps.

References

1. S. Buzzaccaro, E. Secchi and R. Piazza, *Physical Review Letters*, 2013, 111, 048101.